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INVESTIGATING CONSISTENCY AND STABILITY OF TEMPERAMENT IN TWO
STRAINS OF RATTUS NORVEGICUS

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ABSTRACT

Personality (consistency of temperament across conditions) was once thought to be exclusive to humans. It is now, however, recognized in a wide range of non-human animals and is measured using assays of activity and response to novelty. We measured the utility of such temperament assays in *Rattus norvegicus*; the Long-Evans and Sprague-Dawley strain, specifically addressing the effects of enrichment loss and exposure to chronic mild stress. As temperament is, by definition, stable over time and context, we hypothesized that the removal of enrichment and exposure to chronic mild stress (CMS) would not significantly change temperament measures. We used open field and novel object tests, and evaluated three cohorts of rats. Measures from the open field indicating boldness were: high locomotion and time spent in the middle of an arena. Novel object measures reflecting a bold phenotype were: high activity, short latency to reach novel objects, and high proportion of time around novel objects. Each cohort was measured for: baseline scores, after a manipulation to cage enrichment, and the third cohort of rats also experienced CMS (both social and physical stressors) before a further set of assays.

Surprisingly, we found a considerable lack of consistency across the three cohorts studied for both the open field and the novel object assays. Such a lack of predictive value of these tests across time questions the utility of these methods. We therefore suggest that the open field and novel object tasks should be avoided in future rat temperament studies.

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INTRODUCTION

While originally believed to be exclusively human, personality traits are now widely accepted to exist and are extensively studied in non-human animals (referred to as animals hereafter). Personality has been defined in many ways, but it is generally accepted that it encompasses temperaments, character traits, dispositions, goals, attitudes, moods, physical states, and life stories (Gosling, 2001). The difference between personality and temperament, however, is unclear and scientists, psychologists, ecologists, and evolutionary biologists still debate terminology (Allport 1937; Box, 1999; Budaev, 1997; Buss et al., 1987; Clark & Wilson, 1999; Gosling, 2001; Koolhaas et al., 1999; Lowe & Bradshaw, 2001; Pfeffer et al., 2002). Some agree that temperament has a more restricted definition than personality; for example, temperament can be described by differences in emotionality or in traits that are displayed early in life (Box, 1999; Budaev, 1997). In their review, Réale et al. (2007) propose that temperament, personality and individuality are suites of behaviors that are stable across situations and over time (Box, 1999; Budaev, 1997; Dall et al., 2004; Gosling, 2001; Lowe & Bradshaw, 2001). Temperament studies have been used for a variety of purposes, such as measuring the state and welfare of animals (Lansade et al., 2008) and predicting learning and behavior (Görisch & Schwarting, 2006). Knowing the state and welfare of animals is important to help promote the well being of animals, especially domesticated farm animals. The role of temperament in cognition has been of interest in many fields in psychology; particularly as animals with different temperament traits are known to use different learning strategies (Hemsworth et al., 2011). The following review of current literature illustrates how temperament affects many different components of animal behavior and why we need a better understanding of when and how temperament can affect studies of animal behavior.

Typical tests to measure temperament are the open field, novel environment, and social tests. According to Réale et al. (2007), because the open field and novel environment tests are novel situations, the traits that will be exhibited in this type of ecological context are exploration or avoidance (Archer 1973; Hall, 1934). The temperament traits shown during social tests are aggression and sociability (Armitage, 1986). For the open field, the subject is placed into a novel, bare area. As this is a wide space, the animal will feel anxious and will stay near the walls of the arena. A high response, or more active, animal will show less anxiety and so will cross into the middle and be more explorative than a low response, or timid, animal. Similar observations occur in a novel environment test, except that in this instance there are objects in the arena. Typically, activity, latency to approach a novel object/area, and time spent around the novel object/area are measures of exploration or avoidance, with low responding, timid individuals typically taking longer to approach a novel object (Archer 1973; Hall 1934). Social tests introduce the subject to an unfamiliar conspecific, and latency to approach, time spent near, and activity with the conspecific are measures of aggression or sociability (Armitage, 1986).

Previous studies of temperament with many species of animals have described consistent, stable temperament behaviors across time and context (Box, 1999; Budeav, 1997; Dall et al., 2004; Gosling, 2001; Lowe & Bradshaw, 2001). This has been reported for domesticated animals, wild animals, and laboratory animals. Two breeds of *Equus ferus caballus*, Welsh ponies and Anglo-Arabian horses, were measured at three different ages in a novel object test, novel area test, and two surprise tests. The novel object and novel area tests were both types of novel environment tests as outlined above. The two surprise tests were performed while each horse was free in the pen or, alternatively, held by a handler. The surprise was an umbrella opened in front of the test subject. Consistent and correlated behaviors were observed for the

novel object, the novel area, and the surprise test where the horse was not held. These observations were also consistent for all three ages that were tested (Lansade et al., 2008). Together, the results from these tests allowed the horses or ponies to be described as fearful or not fearful.

Temperament in European birds called great tits, *Parus major*, was measured using novel environment, novel object, and novel social tests. Here, the birds were selected for the study based on their lineage. An artificial bidirectional selection program that was based on exploration tests carried out at 35 to 50 days of age selectively bred birds that were fast or slow at exploring. Birds from the third and fourth generation of these fast and slow lines were then screened for their temperament. While the behaviors measured were not consistent over time individually, the selected line differences for slow and fast explorers were apparent at both juvenile and adult phases, showing group differences between fast and slow explorers but not consistent behavior in individuals (Carere et al., 2005). In the red squirrel (*Tamiasciurus hudsonicus*), behaviors were measured with an open field test to quantify activity and an open field with a mirror test to measure aggression. After five minutes in the open field, squirrels remained in the arena for an additional five minutes during which one wall with a mirror was uncovered for mirror-image stimulation. The distance between capture locations and primary location was also measured as an index of boldness and exploration in the natural habitat; the primary location was where the squirrels were placed, and the capture location was where the scientists re-captured squirrels. Larger distance between primary and capture locations correlated with boldness. Maximum capture distance, indicating boldness, correlated with activity and aggression (Boon et al., 2008). Squirrels that travel further in search for food and other resources are more active and are more aggressive presumably because they run the risk of meeting predators as they move around. Less bold and less aggressive individuals are more likely to remain near their primary location and

will likely encounter fewer predators.

Additional studies have found a consistent relationship between temperament and physiology or breeding. The relationship between temperament and immunity was examined by comparing nude mice to hairy mice. The mutation that makes the mice nude and athymic caused lower T-cell-dependent immunity than normal euthymic, hairy mice. Compared to the hairy mice, the nude mice were less active in the open field, and they interacted less with a conspecific, illustrating the correlation between temperament and immunity. Lower immunity was related to lower activity (Vidal 1996).

Lines of first generation reared rainbow trout, *Oncorhynchus mykiss*, bred for high response (HR) and low response (LR) based on post-stress cortisol levels in the parental generation have also been studied (Overli et al., 2002). The trout lines were compared in their response to stress after isolation and after interaction with an “intruder fish” that was a rainbow trout from a different population. Following these tests, half the trout were confined for an hour before cortisol samples were taken. HR fish had higher cortisol levels after confinement compared to LR fish, and HR fish had higher activity than LR fish when tested with an intruder (Overli et al., 2002). Fish that have more active (HR) behaviors have higher stress responses, indicated by increased cortisol levels.

Domesticated Siamese fighting fish, *Betta splendens*, have also been compared to their wild-type counterpart. It was proposed that the domesticated fish would be more aggressive than both their wild-type forms due to selective breeding for fighters in areas such as Thailand where cockfight-like contests occur (Verbeek et al., 2007). Some of the results confirmed this hypothesis; however, as some of the results did not support the hypothesis, the authors suggested that the type of test used in measuring behavior in a specific species should be considered when

studying temperament. Wild-type *B. splendens* and *B. smaragdina* were compared to the domesticated *B. splendens* fighter plakat, *B. splendens* fancy plakat, and *B. splendens* Mahachai. The fish were compared in mirror and video tests that were taken twice, followed by a mutual-viewing test to measure aggression. The fish were first exposed to a mirror, followed by a video of a conspecific. Both the mirror and video tests were equivalent to an intruder test and were supposed to arouse a response, such as aggression, to the conspecific. Finally, the mutual-viewing test had two fish placed in separate tanks next to each other. The results for both the mirror and video tests showed domesticated *B. sp. Mahachai* were more aggressive than wild-type *B. splendens*; however, domesticated *B. splendens* were not more aggressive than wild-type *B. splendens*. Results from the mutual-viewing test showed that domesticated *B. splendens* were more aggressive than wild-type *B. splendens*; no differences were found between wild-type *B. splendens* and *B. sp. Mahachai* (Verbeek et al., 2007). The difference in aggression between domesticated and wild-type *B. splendens* from the mirror and video tests and mutual-viewing test is an example of how different assays can measure different behaviors. If only one of these tests were used, for example the fish were only tested by mirror and video tests, researchers would incorrectly conclude that domesticated and wild-type *B. splendens* are equally aggressive. The type of assay must be carefully considered to draw correct conclusions about an animal's temperament.

This thesis examines the temperament traits of multiple cohorts of the species *Rattus norvegicus*, Long-Evans and Sprague-Dawley strains, to determine if behavior is stable over time and if it is affected by manipulations such as enrichment and chronic mild stress (CMS). It was hypothesized that if the behaviors truly represent temperament, then they would be stable over time in these two strains and whether temperament is affected by manipulations such as

enrichment and CMS. It was hypothesized that if the behaviors truly represent temperament, then they would be stable over time and would not be affected by removal of enrichment or by CMS, or the responses may be shifted from background but similarly so for animals with specific temperaments.

Behavior studies in rats examining temperament have also found consistent group differences within populations. In Wistar strain rats, temperament was measured after neurochemical manipulations. Rats previously classified as high responders, high numbers of rears and high locomotion, habituated to the open field arena after repeated testing. Rats previously classified as low responders did not habituate to the test arena. Habituation was confirmed by the decreased level of rears and locomotion, and with habituation, the activity level of the high responders dropped to equal the activity level of the low responders. Adding a muscarinic antagonist, however, reinstated the group differences in behavior between high and low responders (Thiel et al., 1999). Based on these results, Görisch and Schwarting (2006) examined the relationship between activity and rearing. Using Wistar rats, they found that the amount of rearing in a novel open field corresponded with activity in a plus-maze and memory in a radial maze. Males that reared more also moved around more in the novel open field, and this was also true for the plus-maze. These males also found food pellets at the end of the arms in a radial maze more quickly. The high-rearing rats made fewer reference memory errors (entries into non-baited arms) but more working memory errors (re-entries into baited arms) than the low-rearing rats. (Görisch & Schwarting, 2006). Differences in behavior and cognition between high-rearing and low-rearing rats did not appear to be due to motivational factors; rather, high-rearing rats were more effective in finding bait due to their high amount of activity.

In studies where different strains of rats have been compared, temperament has differed

between strains and individuals within strains (Mällo et al., 2007). Comparing Sprague-Dawley to Wistar rats, behavior has been measured in multiple forms of tests in attempts to capture temperament in these animals. Behavior on the second day of an experiment in an open field-like test was the best predictor for future exploratory activity. In both strains, high exploratory rats were significantly more active than low exploratory rats. In Sprague-Dawley high exploratory rats, activity was reduced by repeated testing but remained higher than that seen in low exploratory rats. Wistar high exploratory rats increased activity in subsequent tests. Social behavior, measured by placing two previously singly-housed rats together in a novel chamber, was not significantly different between high or low exploratory rats or strains, although Sprague-Dawley high exploratory rats had a significantly higher social activity in the second test compared to the first test. The social behavior test could have been insufficient in producing different responses in the high and low exploratory animals; the Sprague-Dawley rats may have become more social with the second test. An exploration effect on the elevated plus-maze and forced swim tests (rats were placed in a cylinder with water for five minutes) were also found; high exploratory rats moved more than low exploratory rats in both tests. High exploratory rats had significantly higher baseline dopamine levels and higher dopamine levels in response to an amphetamine treatment (Mällo et al., 2007). Thus, response to novelty has some basis in brain and hormonal responses, and high exploratory rats are more prone to drug addiction.

Other studies with Sprague-Dawley rats have shown they have stable behavior traits over time that could indicate temperament. In a two-part study, a first cohort of male Sprague-Dawley rats were tested in an exploration arena at 4 months and 8 months old. A second cohort of male Sprague-Dawley rats were tested in the arena at 20 days old, 24 days old, and 11 months old. A square test arena contained three novel objects in three corners and the home base bowl in the

final corner. In the first cohort, more activity correlated with more inspection of the objects, and fearful behavior at 4 months was the same at 8 months. In the second cohort, neophobic infants moved and explored less at 11 months old. (Cavigelli & McClintock, 2003). A further study with male Sprague-Dawley rats looked at their behavior. The animals were tested in a novel non-social arena and novel social arena at two ages (2.5 and 8 months). The novel non-social arena was the same as in Cavigelli & McClintock (2003), and the novel social arena test used the same arena as the novel non-social but with an empty cage and a cage with an unfamiliar male rat of the same age, size, and strain. The latency to approach novelty across these two studies was found to be stable; 19% of the rats were “inhibited,” meaning they displayed fearful behavior in all four tests, and 11% were “non-inhibited;” the rest of the cohort were mixed (Cavigelli et al., 2009). Comparing between the sexes of Sprague-Dawley rats in novel object and novel social arenas, all rats moved more, made more contact with novel, and reared more in social arena, and this was consistent across the sexes. Females were more active in general (Cavigelli et al., 2011).

To test the effect of early social environment on temperament, wild-type Gröningen rats were raised in male-biased or female-biased litters. Temperament in the males was measured with open field, defensive burying, and resident-intruder tests at 90 and 120 days old. There was no effect of rearing condition at day 90, and behavior was not correlated across tests. There were also no consistent behavioral responses to the same test from day 90 and 120 (Gracceva et al., 2011).

Few, if any, temperament studies have been done with the rat strain Long-Evans. There are also very few studies that examine the effect of enrichment or stress on temperament. The effects of enriched condition (EC) in mice from birth have been compared to standard condition (SC) mice, and this was measured using the elevated plus-maze, an open field, and cat odor

exposure tests (Roy et al., 2001). The elevated plus-maze was constructed with two opposing open arms (no walls) and two opposing enclosed arms. In the elevated plus-maze, EC mice entered more enclosed arms more often and performed more head scanning than SC mice. EC mice also stretched less and did not defecate as much as SC mice. The open field was divided into a central area and peripheral area. In the open field, EC mice took less time to leave the initial area in the peripheral area, they had higher peripheral area entries, and they performed less stretching. There were no significant effects of the enrichment rearing conditions for the odor test, but for the mice that experienced the cat odor, the SC mice had higher cortisol levels than EC mice (Roy et al., 2001).

An example of stress affecting temperament can be seen in the Verbeek et al. (2007) study with domesticated *B. splendens*, in which one cohort of fancy plakats was placed in isolation. For thirty minutes a day, the partitions between tanks blocking the fish from seeing each other were removed; at all other times, the fish were isolated. With the mirror and video tests, the isolated plakats showed more aggression than socially reared plakats, but no difference in aggression was seen in the mutual-viewing test (Verbeek et al., 2007). When placed in isolation, fish may develop more aggressive behaviors to conspecifics than socially-reared fish.

As both experience during development and growth can have an effect on adult habits, it would be useful to know how the effect of stress through adolescence affects temperament. One study addressing this tested Wistar and Fawn Hooded rats that were put in social or isolated conditions at 21 days of age and kept this way until experiments were conducted 12 weeks later. These experiments included two variations of open field and of novel object tests and plasma corticosterone measures after open field tests. Rats reared in isolation were tested alone in the open field and socially-reared rats were tested in pairs in the open field prior to the corticosterone

measures. In general, the results showed that rats reared in isolation exhibited less activity than socially-reared rats, but no difference in corticosterone levels was found (Hall et al., 2000).

The aims of this thesis study were to examine the stability of temperament and the effect of enrichment and stress on temperament in two different strains of male rats. Three cohorts of different ages and strains were tested in open field and novel object arenas at multiple time points. Behavior testing at different ages for these strains has not previously occurred, nor has the effect of enrichment or chronic mild stress on behavior been quantified. Cohort 1 used Long-Evans rats that were tested three times from early to late adulthood. Between the first and second tests, the rats underwent cognitive training, and between the second and third tests, enrichment was removed for half of the cohort. Cohort 2 consisted of both Long-Evans and Sprague-Dawley rats. These were tested twice in late adulthood; between the two tests, the rats underwent cognitive training, and half the animals in each strain experienced the removal of enrichment. Cohort 3 were all Long-Evans rats that were tested once during adolescence and once at adulthood. Between the tests, the rats experienced a period of chronic mild stress. It was hypothesized that temperament should be stable and consistent over time, and so the removal of enrichment and the chronic mild stress should not significantly change temperament scores.

METHODS

Sample and Design

Three cohorts of rats were run through an open field and a novel object test at different ages. These tests were conducted in the same room as the rats were housed at Penn State University (South Frear Building, University Park campus). The room was maintained at 22°C and 50% humidity. All rats were supplied by Harlan Laboratories. The rats were housed in pairs in solid-bottom plastic cages (43.5 cm x 23.5 cm x 20.5 cm) and maintained on a 12L: 12D

lighting schedule (lights on at 22:00 h). The rats were on a reversed light-dark schedule because they are nocturnal, so we needed their active period to be during the day for testing. Food and water were available *ad libitum*, and cages were changed once a week by animal facility personnel. The rats were handled daily throughout the study. When used, the enrichment items in each cage were two blocks of 1 in. x 3 in. cedar sticks and two white, polyvinyl chloride (PVC) tubes with 3 in. diameter that were attached to the top of the cage. The health status of the cohorts was monitored by testing for 14 common pathogens in ‘sentinel’ rats regularly exposed to soiled bedding of all cohort rats; none of these pathogens were identified in the sentinel rats.

During testing, the room was lit with three 40W red bulbs that were pointed away from the testing arena. The low lighting was designed to provoke minimal anxiety in the animals. Each test for each rat was recorded on DVD, and the data were collected from these recordings. To try to avoid the variance due to circadian rhythms of stress hormones, testing was performed during the same time of day for all groups and all tests. Each test was performed at approximately 13:00 h, three hours after lights out, which is in the middle of the rats’ active period (Cavigelli et al., 2011). For each temperament assay, the order of rats was randomized to control for order effects and potential time of trial within the testing window. Because the rats were pair-housed, the animal from the pair that ran through the tests first was randomized between cages. All methods were approved by The Pennsylvania State University Institute for Animal Care and Use Committee (IACUC #35761).

Open Field Test

The open field test was conducted in a square arena (120 cm x 120 cm x 46 cm) made of white opaque plastic, similar to the arena used in Cavigelli et al. (2011). Before the test, the individual being tested was removed from its cage and placed in an empty cage for thirty seconds

to acclimate (Hall et al., 2000). Then, the rat was carried and placed along the edge of the arena in the middle of one wall, facing the right side. The observer left the room after the rat was placed in the arena. Each rat was left in the arena for five minutes, during which time the rat could roam and explore freely. The five-minute trial began when the observer's hands left the rat; after the trial, the observer re-entered the room to retrieve the animal. A five-minute trial was chosen based on previous studies' methods (Cavigelli & McClintock, 2003; Cavigelli et al., 2011; Cavigelli et al., 2009; Hall et al., 2000). After each test, the rat was returned to its cage, and the arena was sprayed with 70% ethanol and dried with paper towels to remove the odor cues left by the rats.

Once the trials were completed, the DVD's were replayed, and the behavior was quantified. The data collected were time at the edge, first time to cross into the middle, the total number of crosses into the middle, the total number of squares crossed on a superimposed 8 x 8 grid, and the number of fecal boli each rat produced as a proxy of stress. During video analysis, a transparent grid was placed over the computer screen; this divided the arena into 64 equally sized squares. The edge was defined as the single outermost squares lined against the wall; the middle was the area within the edge. Movement across a square was defined by an animal's entire body (all four limbs) crossing into the next square.

Novel Object Test

The novel object test was conducted in the same square arena as the open field test, 24 hours after the open field test. The novel objects used for the first set of tests were a red rubber bowl (3.5 in. diameter) and a transparent square tube (15 cm long). The objects for the second test were a large silver bowl and a red, half trapezoid with the open side facing the floor for Cohorts 1 and 3. Cohort 2 had the large silver bowl and a dark blue igloo-shaped object. In the

third test for Cohort 1, the objects were the igloo and a transparent yellow, curvy tube. The objects were placed 15 cm from the corners, one on each side. The home base was located in the middle of the wall furthest from the novel objects. This was called the home base because it was where the animal started the test and it was a place for the animal to go to avoid any choices and forced exploration. The home base was a cylindrical pipe (15 cm long) that was held in place with a block of wood (7.5 cm x 2 cm x 2 cm).

The focal rat was removed from its cage and placed into another cage with the home base pipe for thirty seconds. If the rat entered the pipe by the end of the thirty seconds, it was transported in the pipe to the arena and placed between the wood and the wall with the rat facing the right side of the arena. If the rat did not enter the tube, the tube was placed in the arena, and the rat was placed into the arena facing the tube. Each test's start time began when the observer's hands left the tube and ended after five minutes, after which the observer re-entered the room. After each test, the rat was returned to its cage. The arena, objects, and home tube were sprayed with 70% ethanol and wiped with paper towels after each rat.

The DVD's were replayed after the trials were completed, and the behaviors were measured. The data collected were latency to leave home base, to reach objects, and to return to home base; the percentage of time spent within one body length of object 1, object 2, and home base; the number of rears; the total number of squares crossed; and the number of fecal boli. The percentage of time around an object was defined as when the animal was within one body length and facing the object. As conducted for the open field, a transparent grid of 8 x 8 squares was placed over the computer screen for video analysis.

Cohort 1

The first cohort consisted of outbred male Long-Evans rats (n=12). Three days after their

arrival, enrichment was added to their cages (see *Figure 1*). A week later, at 69 days of age, each rat was run through an open field test. The following day, during the same time period, the animals underwent a novel object test. The animals were also used in a cognitive training study before their next set of temperament tests.

The second open field and novel object tests for Cohort 1 occurred at 174 and 175 days of age. Following these, enrichment was removed from half of the cohort by housing them in standard cages without enrichment items, and the rats continued with behavior tasks. The third set of tests occurred at 189 and 190 days of age with half of the cohort retaining enrichment and the other half without enrichment.

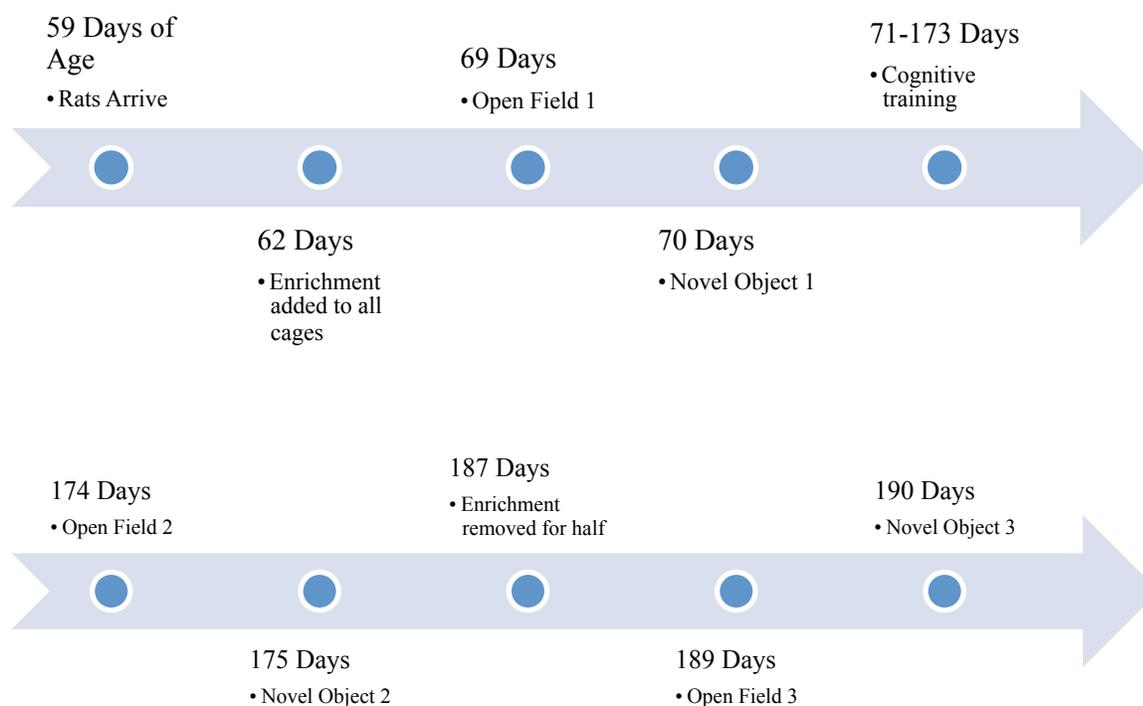


Figure 1. Timeline of temperament testing for Cohort 1.

Cohort 2

Cohort 2 comprised of 6 Long-Evans and 6 Sprague-Dawley outbred male rats (n=12).

The Long-Evans rats were born 6 days earlier than the Sprague-Dawley rats. After arriving, the animals were given a week to acclimate (see *Figures 2, 3*). Enrichment items were added at the end of the week of acclimation (175 days of age for Long-Evans; 181 days of age for Sprague-Dawley). A week after enrichment was added to their cages, rats were run through the open field and novel object tests on consecutive days (182 and 183 days of age for Long-Evans; 188 and 189 days of age for Sprague-Dawley). The cohort went through cognitive training; then, enrichment was removed from the cages of 4 Sprague-Dawley rats and 2 Long-Evans rats. This was done to evenly distribute the effect of loss of enrichment across the 12 rats in the cohort. It could not be 3 Sprague-Dawley and 3 Long-Evans because the rats were pair-housed by strain. The second set of tests was conducted with half of the cohort retaining enrichment and the other half in a standard cage with no enrichment (263 and 264 days of age for Long-Evans; 269 and 270 days of age for Sprague-Dawley).

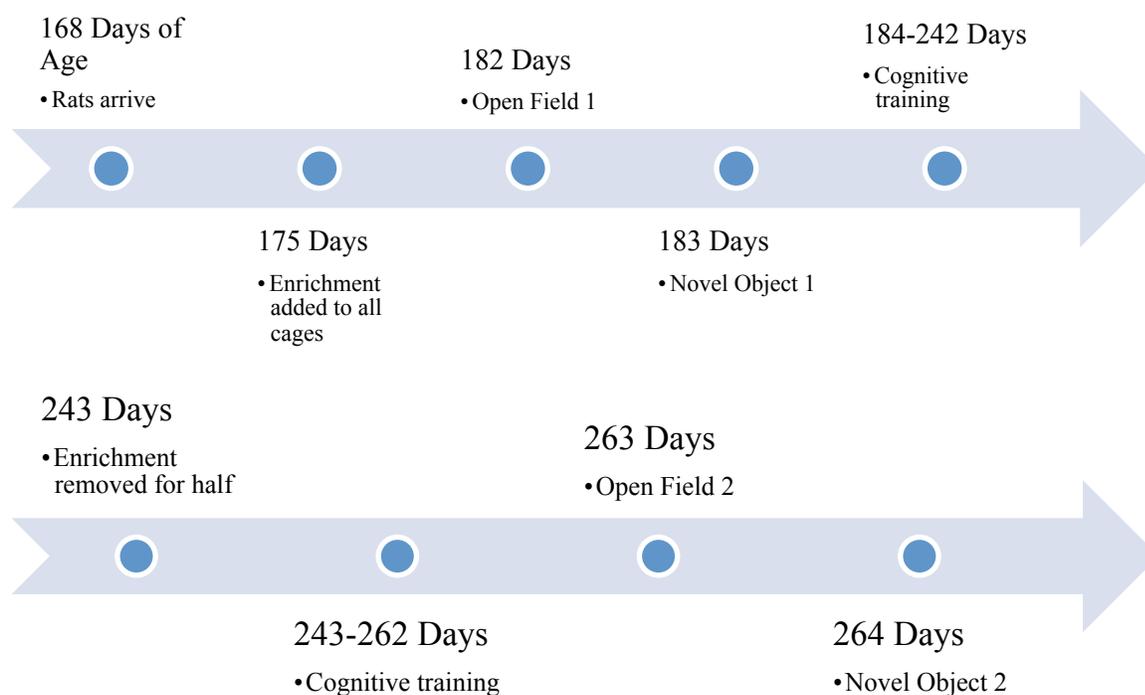


Figure 2. Timeline of temperament testing for the Long-Evans rats in Cohort 2.

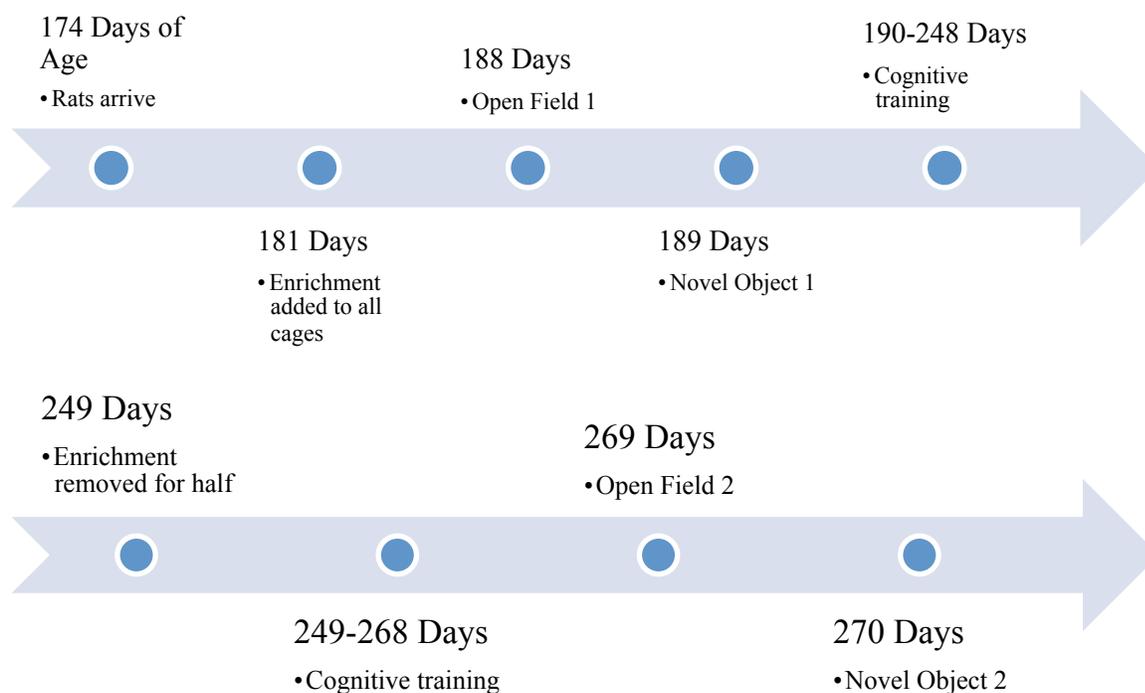


Figure 3. Timeline of temperament testing for the Sprague-Dawley rats in Cohort 2.

Cohort 3

Cohort 3 had outbred Long-Evans male rats ($n=16$). Each rat went through an open field and novel object test starting at age 29 and 30 days, respectively, a week after their arrival (see *Figure 4*). Half of the cohort experienced chronic mild stress from day 35 to day 76. The chronic mild stress incorporated a combination of social and physical stressors, including damp bedding, cage tilt, small cage, crowding, isolation, and foreign bedding. At 84 and 85 days old, each rat was run through the open field and novel object tests for a second time.

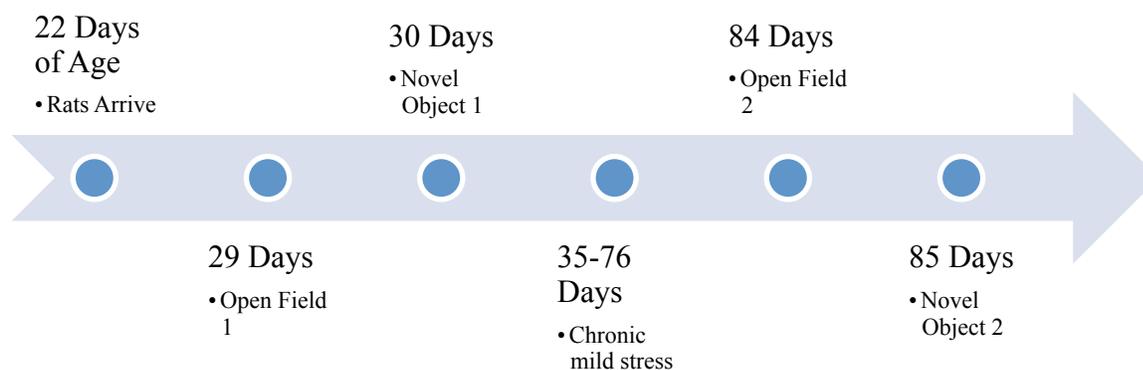


Figure 4. Timeline of temperament testing for Cohort 3.

Data Collection and Analyses

All data were collected by watching recordings and timing with a stopwatch. To count the number of squares crossed at the edge in the open field test, a transparent copy was used to overlay the computer screen. The copy had a grid that split the arena into 8x8 squares. The graphs were made using Microsoft Excel. StatView (1997) was used to run the data analyses, using correlation z-tests, paired t-tests, and repeated measures ANOVA. All data were tested for homogeneity of variance using the equality of variances f-test. Where necessary, the data were transformed using log transformations. These were the checks for equality of variance before the ANOVAs were run.

RESULTS

With three cohorts, three hypotheses were addressed for the results. These were: i) temperament should be stable and consistent over time; ii) the removal of enrichment should not significantly change temperament; iii) the chronic mild stress (CMS) should not significantly change temperament. Previous studies (Boon et al., 2008; Carere et al., 2005; Cavigelli et al., 2011; Görisch & Schwarting, 2006; Gracceva et al., 2011; Mällo et al., 2007; Overli et al., 2002; Thiel et al., 1999; Vidal, 1996) have typically focused on activity or locomotion to score

temperament, measured by the total number of squares crossed in both the open field and novel object tests and latency to the first object in the novel object test. This was also true here, as the extra measures taken from the DVD recordings produced no significant or consistent results.

Temperament Consistency across Time

There were no significant correlations for Cohort 1 for the first and second temperament assays. (Total number of squares crossed in the open field [$r=.372$; $p=.2414$]; total number of squares crossed in the novel object tests [$r=.049$; $p=.8823$]; latency to the first object did not correlate [$r=.033$; $p=.9211$]).

Effect of Loss of Enrichment on Temperament

Cohorts 1 and 2 both experienced losses of enrichment, with half of the animals losing enrichment items before undergoing the temperament assays. There were no significant results for Cohort 1. The ANOVA for the total number of squares crossed in the open field did not reveal significant results [$F(1,10)=.172$; $p=.6868$]. (Total number of squares crossed in the novel object test showed no significant effect of enrichment [$F(1,10)=.109$; $p=.7481$]. Latency to the first object also showed no effect [$F(1,10)=.001$; $p=.9710$]).

For Cohort 2, however, a two-factor ANOVA with repeated measures showed a significant effect of enrichment on the total number of squares crossed in the open field [$F(1,8)=5.432$; $p=.0481$]. There was also a significant effect of strain on the total number of squares crossed in the open field [$F(1,8)=14.696$; $p=.005$]. As seen in *Figure 5*, the animals that lost enrichment moved more in the second test, and the Sprague-Dawley rats moved more than the Long-Evan rats. There was also a significant Enrichment X Strain interaction [$F(1,8)=9.499$; $p=.0151$]. The Sprague-Dawley rats that lost enrichment had the highest amount of activity. For the total squares crossed in the novel object test, there was a significant effect of enrichment

[$F(1,8)=8.686$; $p=.0185$]. As shown in *Figure 6*, the animals that lost enrichment moved more in the second test. The ANOVA for the data for the latency to the first object did not reveal any significant effects (enrichment [$F(1,8)=.167$; $p=.6934$]; strain [$F(1,8)=.789$; $p=.4004$]; enrichment X strain [$F(1,8)=1.202$; $p=.3049$]).

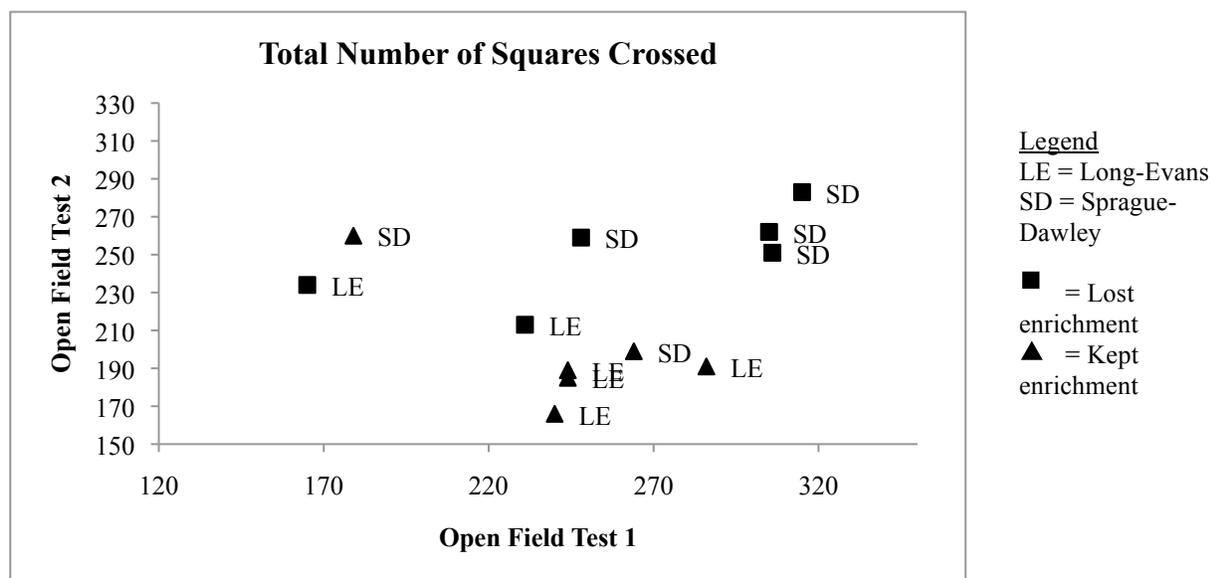


Figure 5. Total number of squares crossed in the first open field test compared to the second open field test. Before the second test, the Long-Evans (LE) and Sprague-Dawley (SD) strains remained enriched or lost enrichment. There was a significant effect of enrichment and a significant effect of strain. The animals that lost enrichment moved more in the second test, and SD rats moved more than LE rats. Together, there was a significant Enrichment X Strain interaction. The SD rats that lost enrichment had the highest amount of activity.

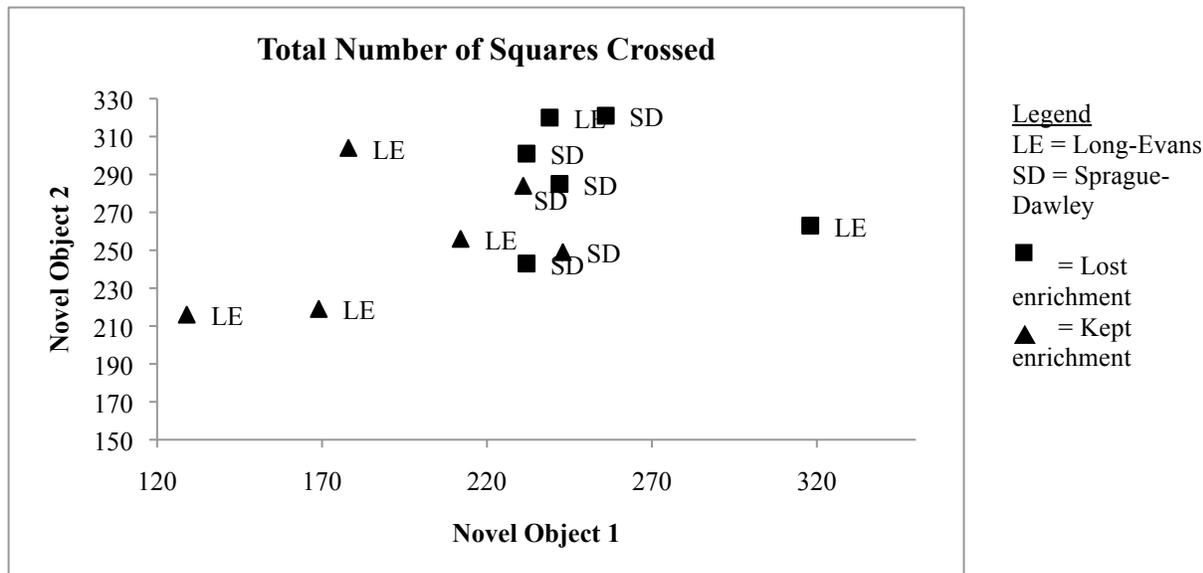


Figure 6. Total number of squares crossed in the first novel object test compared to the second novel object test. Before the second test, the Long-Evans and Sprague-Dawley strains remained enriched or lost enrichment. There was a significant effect of enrichment; the animals that lost enrichment moved more in the second test.

Effect of CMS on Temperament

Half of Cohort 3 experienced CMS after the first set of temperament assays and before the second set of assays. Paired t-tests showed that there were significant differences between sets of tests in both CMS and non-CMS groups. There were differences in the total number of squares crossed in the open field tests for both the CMS group [$p=.0074$] and the non-CMS group [$p=.0337$]. There was a difference in the total number of squares crossed in the novel object tests for the non-CMS group only [$p=.0316$]. There were no differences for the latency to the first object for either group.

The ANOVA for the total number of squares crossed in the open field did not reveal a significant effect of CMS [$F(1,14)=.192$; $p=.6680$]. *Figure 7* shows the raw data; it appeared that the CMS group had values closer to the mean than the non-CMS group. For the total number of squares in the novel object, there was no significant effect of CMS [$F(1,14)=.022$; $p=.8832$]. The raw data is shown in *Figure 8*; as in *Figure 7*, it appeared that the CMS group had more values in

the middle, with the non-CMS group having more extreme values. The latency to the first object showed no significant effect of CMS [$F(1,14)=1.544$; $p=.2345$].

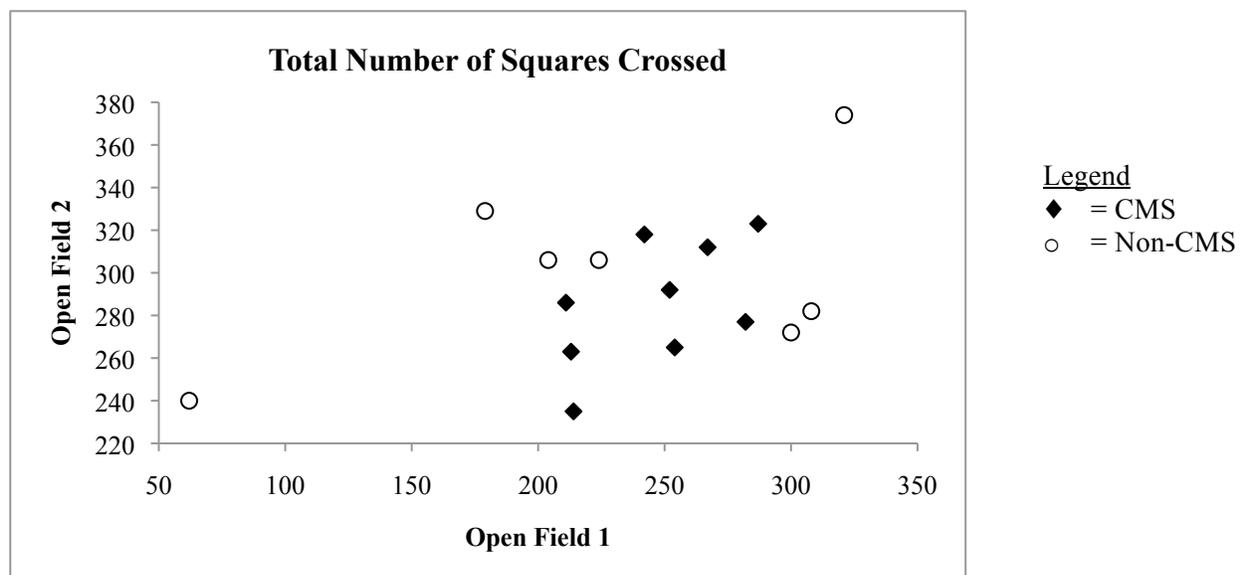


Figure 7. Total number of squares crossed in the first open field test compared to the second open field test. There was no significant effect of CMS, but it appeared that the CMS group had values closer to the mean than the non-CMS group.

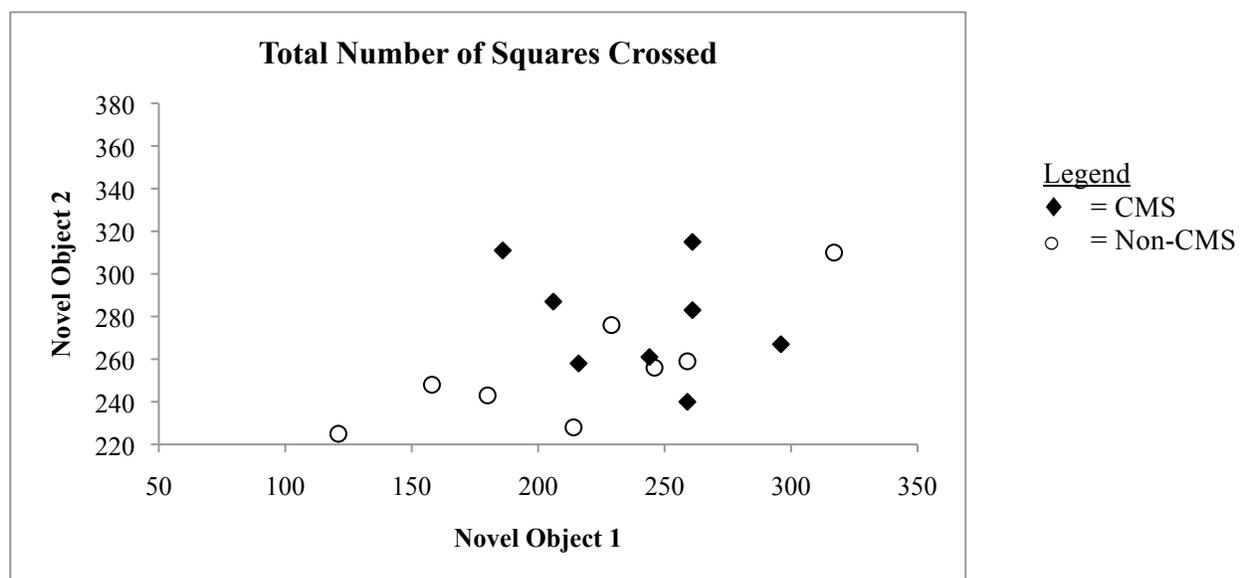


Figure 8. Total number of squares crossed in the first novel object test compared to the second novel object test. There was no significant effect of CMS, but it appeared that the CMS group had more values in the middle, and the non-CMS group had more extreme values.

DISCUSSION

The purpose of this thesis study was to examine the consistency of temperament and the effects of enrichment and chronic mild stress on temperament in two strains of rats.

Temperament behaviors have been observed in horses, great tits, red squirrels, mice, rainbow trout, and Siamese fighting fish (Boon et al., 2008; Carere et al., 2005; Lansade et al., 2008; Overli et al., 2002; Verbeek et al., 2007; Vidal 1996). There has been evidence of temperament in rats as well; Wistar, Sprague-Dawley, Gröningen, and Fawn Hooded rat strains have exhibited behaviors suggesting temperament in rats (Cavigelli & McClintock, 2003; Cavigelli et al., 2009; Görisch & Schwarting, 2006; Graceva et al., 2011; Hall et al., 2000; Mällo et al., 2007.) Based on the results of these studies and the definition of temperament (Box, 1999; Budeav, 1997; Dall et al., 2004; Gosling, 2001; Lowe & Bradshaw, 2001), it was hypothesized that temperament would be stable across time and that enrichment removal or CMS would not significantly affect temperament behaviors.

The rat test animals were measured at multiple time points with two temperament assays, open field and novel object, each time (see *Figures 1-4*). As seen by Cohort 1's results comparing the first and second set of tests, the behaviors exhibited in the open field and novel object tests were not consistent from time point one to time point two. There were no significant correlations between the first and second set of assays for the measures of total number of squares in the open field and novel object and of latency to the first object in the novel object. Thus, the hypothesis that temperament would be stable across time was not supported.

The effect of loss of enrichment was studied in Cohorts 1 and 2. The results from Cohort 1 showed no effect of enrichment. For Cohort 2, there were strain and enrichment loss effects for certain measures. In the open field, the Sprague-Dawley rats that lost enrichment had the highest

amount of locomotion compared to the Long-Evans rats and rats that kept enrichment. In the novel object trials, rats that lost enrichment also had higher locomotion than rats that kept enrichment. These results differ from previous results found for enriched (lived in groups of 10; larger cages with enrichment items) versus standard conditioned (lived in groups of 3-4; standard cages) mice; enriched mice displayed higher activity (Roy et al., 2001). In this thesis, there were no significant results for the latency to the first object from the novel object assay. This contradicts previous results describing the behavior of Sprague-Dawley rats where activity positively correlated with inspection of novel objects in the arena (Cavigelli & McClintock, 2003). The results found in this thesis, however, do not support the hypothesis that enrichment removal would not significantly affect temperament.

Cohort 3 experienced chronic mild stress (CMS) between the first and second set of temperament tests; there were differences in the sets of data as seen in the paired t-tests, but the ANOVA did not reveal an effect of the CMS. This result contrasts with those found in a previous study examining behavior after isolation. In Wistar and Fawn Hooded rats, isolated subjects exhibited less activity than socially-reared subjects (Hall et al., 2000). As there were differences between the first and second set of tests, although they were not due to CMS, the hypothesis that temperament is stable across context was also not supported.

The results from each cohort were unexpected and did not support the proposed hypotheses. Possible reasons why the results were inconsistent with the hypothesis include the Long-Evans strain of the animals, laboratory conditions, and/or methods. Few temperament studies have been conducted with Long-Evans rats; most have used Wistar, Fawn Hooded, and Sprague-Dawley rat strains (Cavigelli & McClintock, 2003; Cavigelli et al., 2011; Cavigelli et al., 2009; Görisch & Schwarting, 2006; Hall et al., 2000; Mällo et al., 2007). It is possible that

Long-Evans rats just do not exhibit stable temperament behaviors in any type of temperament assay. Typically, Long-Evans rats are used for diet-induced obesity, nutrition, and behavior studies (“Long Evans,” 2012). Given this sensitivity to dietary conditions, Long-Evans rats may be behaviorally flexible and may not display any single temperament behavior when placed in a testing arena.

Another possible reason for the inconsistencies could be the conditions of the lab. and testing room. Owing to space constraints in the animal facility, the temperament assays were carried out in the room the animals were housed in, not in a separate room as previous studies had done (Cavigelli & McClintock, 2003; Cavigelli et al., 2011; Cavigelli et al., 2009; Görisch & Schwarting, 2006; Hall et al., 2000; Mällo et al., 2007; Roy et al., 2001). Noises and smells from the cages could well have affected the test subject’s behavior. Also, noises caused by caretakers or other people passing by the testing/housing room could not be controlled, and these could have also affected rat behavior. How soon testing occurred after cage changes, who the tester was, and other stressful events could further have had an effect on the temperament testing. While certain conditions could not be controlled, it is recommended that future testing be carried out in a separate room from the colony room and at times of day when there are likely to be fewer disturbances from the animal facility staff.

A further potential reason for the inconsistencies in temperament could be the methods that were employed. This includes sample size, testing arenas, amount of time for each test, and the age of the rats. All three cohorts had small sample sizes compared to previous studies where over 20 subjects for each experiment group were tested (Boon et al., 2008; Carere et al., 2005; Cavigelli & McClintock, 2003; Cavigelli et al., 2011; Cavigelli et al., 2009; Görisch & Schwarting, 2006; Lansade et al., 2008; Mällo et al., 2007; Overli et al., 2002; Roy et al., 2001;

Thiel et al., 1999). To explore this further, the controls of each cohort could have been pooled together for data analysis, but the validity of this approach is questionable since each cohort had different life experiences and was tested at different ages. With three small sample sizes, there was a small distribution of behaviors. Usually only the tail ends of large distributions exhibit explicit temperament traits such as high and low exploratory tendencies. An example of this was seen in Cavigelli et al. (2009), in which out of 53 male Sprague-Dawley rats, 10 were classified as “inhibited” (consistently low activity) and 6 were “non-inhibited” (consistently high activity). The remainder had mixed behaviors. With a smaller sample size, as in the current study, there is less of a chance of consistently different phenotypes.

Some studies that had smaller sample sizes similar to the size of the three cohorts used in this current study have found temperament traits. Verbeek et al. (2007) found significant results with samples of 4, 8, 9, 10, and 13 Siamese fighting fish. Effects may have been seen in these small samples sizes due to fish selectively bred to win cockfight-like contests (Verbeek et al., 2007). Graceveva et al. (2011) also had samples of 9 and 10 wild-type Gröningen rats, and Hall et al. (2000) had samples of 6, 8, and 10 Fawn Hooded and Wistar rats. The wild-type Gröningen strain has an established adult personality structure based on data from previous studies, and the strain has large individual variation (De Boer et al., 2003; Koolhaas et al., 1999; Koolhaas et al., 2006; Koolhaas et al., 2007; Koolhaas et al., 2010); thus, this may be why Graceveva et al. (2011) were able to find consistent behavior within individuals. There have been many studies with Fawn Hooded and Wistar rats, which also could explain why Hall et al. (2000) were able to find significant effects (Hall et al., 1998; Holson et al., 1986; Holson et al., 1991; Overstreet et al., 1996). Therefore, it is possible that temperament behavior is more obvious in certain species or possibly, certain rat strains.

In terms of other physical differences in the test environment that may affect rat behavior, testing arenas vary from study to study. The arena used for the open field and novel object tests in this thesis was made of white opaque plastic. Contrary to previous studies, the floor of the arena was bare instead of covered in bedding similar to what is used in cages, and there was no transparent lid on the arena during the tests (Cavigelli & McClintock, 2003; Cavigelli et al., 2009; Cavigelli et al., 2011). Both the bedding and the lid are used to reduce anxiety in the subject, so the anxiety levels of the cohorts during testing could have affected the results.

The length of the each test was only 5 minutes. This amount of time has been used in mice (Roy et al., 2001), Wistar and Fawn Hooded rats (Hall et al., 2000), and Sprague-Dawley rats (Cavigelli & McClintock, 2003; Cavigelli et al., 2009; Cavigelli et al., 2011). Other studies have used 10 minutes for Wistar rats (Thiel et al., 1999; Görisch & Schwarting, 2006), great tits (Carere et al., 2005) and wild-type Gröningen rats (Gracceva et al., 2011). Still others have used 15 minutes for rainbow trout (Overli et al., 2002) and 20 minutes for Siamese fighting fish (Verbeek et al., 2007). Clearly, length of test has not been standardized. Perhaps with the small sample size, a longer testing time would have revealed more stable and consistent behaviors.

Lastly, the age of the rats may have had an effect on the results. The rats that underwent a CMS treatment were first tested during adolescence, a period of extensive developmental changes (McCormick, 2010). Any behaviors shown in the first set of tests could thus be different than behaviors seen at adulthood due to individual developmental changes. In the second cohort where half the animals were Long-Evans rats and the other half were Sprague-Dawley rats, the rats arrived at 6 months of age. Prior to their arrival, the rats were proven breeders. Whatever life events they had experienced could well have affected how they behaved in the temperament assays.

In conclusion, the hypotheses that temperament would be stable across time and that loss of enrichment or CMS would not significantly affect temperament behaviors were not supported. The causes for this were most likely the lab conditions, sample size, and testing arena. Consistent temperament behaviors have been seen in many species and strains of rats. Thus, it is likely that Long-Evans rats also have temperaments, but they were not resolved in the current study due to issues described above. Future experiments should conduct tests in a room separate from the housing room in an area with minimal noise. The same tester should conduct the tests to control for possible aversive reactions to different people. The sample size should be increased to at least 20 rats for each experimental group (i.e. 20 control and 20 CMS rats). Also, the arena should have bedding on the floor and a transparent lid to decrease anxiety in the subjects. Ideally, the animals would be tested longitudinally: soon after birth, during adolescence, and multiple times during adulthood. This would show true stability and consistency of temperament traits.

REFERENCES

- Allport, G. W. (1937). *Personality: A Psychological Interpretation*. Henry Holt, New York.
- Archer, J. (1973). Tests for emotionality in rats and mice: a review. *Animal Behaviour* **21**, 205-235.
- Armitage, K. B. (1986). Individuality, social behavior, and reproductive success in yellow-bellied marmots. *Ecology* **67**, 1186-1193.
- Boon, A. K., Réale, D., & Boutin, S. (2008). Personality, habitat use, and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus*. *Oikos* **117**, 1321-1328.
- Box, H. O. (1999). Temperament and socially mediated learning among primates. In *Mammalian Social Learning: Comparative and Ecological Perspectives* (eds. H. O. Box and K. R. Gibson), pp. 33-56. Cambridge University Press, Cambridge.
- Budaev, S. V. (1997). "Personality" in the guppy (*Poecilia reticulata*): A correlational study of exploratory behavior and social tendency. *Journal of Comparative Psychology* **111**, 39-411.
- Buss, A. H., Chess, S., Goldsmith, H. H., Hinde, R. A., McCall, R. B., Plomin, R. Rothbart, M. K., & Thomas, A. (1987). What is temperament: Four approaches. *Child Development* **58**, 505-529.
- Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M., & Groothuis, T. G. G. (2005). Personalities in great tits, *Parus major*: Stability and Consistency. *Animal Behaviour* **70**, 795-805.
- Cavigelli, S. A. & McClintock, M. K. (2003). Fear of novelty in infant rats predict adult

- corticosterone dynamics and an early death. *Proceedings of the National Academy of Sciences* **100**(26), 16131-16136.
- Cavigelli, S. A., Michael, K. C., West, S. G., & Klein, L. C. (2011). Behavioral responses to physical vs. social novelty in male and female laboratory rats. *Behavioural Processes* **88**, 56-59.
- Cavigelli, S. A., Ragan, C. M., Michael, K. C., Kovacsics, C. E., & Bruscke, A. P. (2009). Stable behavioral inhibition and glucocorticoid production as predictors of longevity. *Physiology & Behavior* **98**, 205-214.
- Clark, L. A. & Wilson, D. (1999). Temperament: A new paradigm for trait psychology. In *Handbook of Personality: Theory and Research, 2nd edn.* (Eds. L. A. Pervin and O. P. John), pp. 399-423. The Guilford Press, New York.
- Dall, S. R. X., Houston, A. I. & McNamara, J. M. (2004). The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* **7**, 734-739.
- De Boer, S. F., van der Vegt, B. J., & Koolhaas, J. M. (2003). Individual variation in aggression of feral rodent strains: A standard for the genetics of aggression and violence? *Behavior Genetics*, **33**(5), 485-501.
- Görisch, J. & Schwarting, R. K. W. (2006). Wistar rats with high versus low rearing activity differ in radial maze performance. *Neurobiology of Learning and Memory* **86**, 175-187.
- Gosling, S.D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin* **127**(1), 45-86.
- Gracceva, G., Koolhaas, J. M., & Groothuis, T. G. G. (2011). Does the early social environment affect structure and consistency of personality in wild-type male rats? *Developmental*

- Psychobiology* **53**, 614-623.
- Hall, C. S. (1934). Emotional behaviour in the rat. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology* **18**, 385-403.
- Hall, F. S., Huang, S., Fong, G. W., Pert, A., & Linnoila, M. (1998). Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. *Psychopharmacology* **139**, 203-209.
- Hall, F. S., Huang, S., Fong, G. W., Sundstrom, J. M., & Pert, A. (2000). Differential basis of strain and rearing effects on open-field behavior in Fawn Hooded and Wistar rats. *Physiology and Behavior* **71**, 525-532.
- Hemsworth, P. H., Smith, K., Karlen, M. G., Arnold, N. A., Moeller, S. J., & Barnett, J. L. (2011). The choice behavior of pigs in a Y maze: Effects of deprivation of feed, social contact and bedding. *Behavioural Processes* **87**(2), 210-217.
- Holson, R. R. (1986). Feeding neophobia: a possible explanation for the differential maze performance of rats reared in enriched or isolated environments. *Physiology & Behavior* **38**, 191-201.
- Holson, R. R., Scallet, A. C., Ali, S. F., & Turner, B. B. (1991). "Isolation stress" revisited: Isolation-rearing effects depend on animal care methods. *Physiology & Behavior* **49**, 1107-1118.
- Koolhaas, J.M., De Boer, S.F., & Buwalda, B. (1999). Stress and adaptation toward ecologically relevant animal models. *Current Directions in Physiological Science* **15**(3), 109–112.
- Koolhaas, J. M., De Boer, S. F., & Buwalda, B. (2006). Stress and adaptation toward ecologically relevant animal models. *Current Directions in Psychological Science* **15**(3), 109–112.

- Koolhaas, J. M., De Boer, S. F., Buwalda, B., & Reenen, K. (2007). Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain, Behaviour and Evolution* **70**, 218–226.
- Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology* **31**(3), 307–321.
- Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. & Blokhuis, H. J. (1999). Coping style in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Review* **23**, 925-935.
- Lansade, L., Bouissou, M., Erhard, H. W. (2008). Fearfulness in horses: A temperament trait stable across time and situations. *Applied Animal Behaviour Science* **115**, 182-200.
- “Long-Evans (Blue Spruce)” (2012). Harlan Laboratories. Retrieved from http://www.harlan.com/products_and_services/research_models_and_services/research_models/long_evans_blue_spruce_outbred_rat.hl.
- Lowe, S. E. & Bradshaw, J. W. S. (2001). Ontogeny of individuality in the domestic cat in the home environment. *Animal Behaviour* **61**, 231-237.
- Mällo, T., Alttoa, A., Kõiv, K., Tõnissaar, M., Eller, M., & Harro, J. (2007). Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behavioural Brain Research* **177**, 269-281.
- McCormick, C. M. (2010). An animal model of social instability stress in adolescence and risk for drugs of abuse. *Physiology & Behavior* **99**(2), 194-203.

- Overli, O., Pottinger, T. G., Carrick, T. R., Overli, E., & Winberg, S. (2002). Differences in behaviour between rainbow trout selected for high- and low-stress responsiveness. *The Journal of Experimental Biology* **205**, 391-395.
- Overstreet, D. H., & Rezvani, A. H. (1996). Behavioral differences between two inbred strains of Fawn-Hooded rat: A model of serotonin dysfunction. *Psychopharmacology* **128**, 328-330.
- Pfeffer, K., Fritz, J. & Kotrschal, K. (2002). Hormonal correlates of being an innovative greylag goose, *Anser anser*. *Animal Behaviour* **63**, 687-695.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemase, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* **82**, 291-318.
- Roy, V., Belzung, C., Delarue, C., & Chapillon, P. (2001). Environmental enrichment in BALB/c mice: Effects in classical tests of anxiety and exposure to a predatory odor. *Physiology and Behavior* **74**, 313-320.
- Thiel, C. M., Müller, C. P., Huston, J. P., & Schwarting, R. K. W. (1999). High versus low reactivity to a novel environment: Behavioural, pharmacological and neurochemical assessments. *Neuroscience* **93**(1), 243-251.
- Verbeek, P., Iwamoto, T., & Murakami, N. (2007). Differences in aggression between wild-type and domesticated fighting fish are context dependent. *Animal Behaviour* **73**, 78-83.
- Vidal, J. (1995). Differences of nu/+ and nu/nu mice in some behaviors reflecting temperament traits. *Physiology and Behavior* **59**(2), 341-348.

APPENDIX

IACUC Approval of Protocol IACUC #35761

Date: March 16, 2012

From: William G. Greer, Assistant Director, Animal Care, Biosafety and Radiation Programs

To: Victoria A. Braithwaite-Read

Subject: Results of IACUC Protocol Review – Modification (**IACUC# 35761**)

Approval Expiration Date: November 29, 2012

(Note: This date reflects the anniversary date of the actual submission approval date.)

“Cognitive Bias and the Impact of Emotion on Health and Behavior”

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your modification regarding the above referenced protocol.

Comment: Request to change blood collection technique from tail tip amputation to saphenous vein blood collection

This Institution has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare. The Assurance number is A 3141-01. The Pennsylvania State University is also registered with the US Department of Agriculture (Certificate No. 23-R-0021). As of February 13, 2001, The Pennsylvania State University was awarded Full Accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

The IACUC does not require the principal investigator to provide copies of permits (e.g., PA Game Commission, Bird Banding, US Fish and Wildlife Service) prior to approval. However, if your research mandates a permit requirement, it is your responsibility to acquire such permits prior to conducting the research described in your IACUC protocol.

By accepting this decision, you agree to notify the Office for Research Protections of (1) any additions or procedural changes that modify the animals’ risks in any way and (2) any unanticipated study results that impact the animals. Prior approval must be obtained for any planned changes to the approved protocol. Any unanticipated pain or distress, morbidity or mortality must be reported to the attending veterinarian and the IACUC.

On behalf of the IACUC and the University, I thank you for your efforts to conduct your research in compliance with the federal regulations that have been established for the protection of animals.

If you are interested in subscribing or being removed from ORP listserv, send an email to: L-ORP-Research-L-subscribe-request@lists.psu.edu to subscribe or L-ORP-Research-L-unsubscribe-request@lists.psu.edu to unsubscribe. There is no need to add any text in the subject line or in the message body of the email.

WGG/mpp

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